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TITLE: TGFB1 Regulation of Matrix Metalioproteinase-9 in Human Prostate Cancer Metastasis

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13. ABSTRACT *(Maximum 200 Words)*

Matrix Metalloproteinases (MMPs) facilitate invasion, growth factor activation, and angiogenesis during the metastasis of prostate cancer and they have become a popular target for anti-metastasis agents. Existing anti-MMP drugs are synthetically designed pharmacologic antagonists with a relatively broad spectrum of action and clinical toxicities which have proven unacceptable. This New Investigator research pursues an alternative approach to these toxic chemicals. We focus on one MMP--MMP-9 because it is clinically linked with prostate cancer metastasis and can be induced from very low to high levels in prostate cancer cells by TGFβ1, which is itself clinically and experimentally associated with prostate cancer metastasis. We are attacking the proteinase at the level of transcription. Transcriptional upregulation of MMP-9 by TGFβ involves an enhancement of the stability of the MMP-9 mRNA molecules. Understanding how this increased mRNA stability occurs is our project's goal. We are attempting to identify new targets on the MMP-9 transcript protected by the induction or repression of RNA-binding proteins induced by TGFβ1. We are also attempting to isolate and attempt to identify these TGFβ-regulated proteins.

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Introduction

The purpose of this New Investigator grant is to target matrix metalloproteinases that abet prostate cancer metastasis. Our approach is to attack the proteinase MMP-9 at the level of transcription by understanding that in prostate cancer cells, this important enzyme is in part upregulated by the cytokine TGFβ1. This upregulation involves enhancing the stability of the MMP-9 mRNA molecules. We proposed to identify targets on the MMP-9 transcript which may be protected by the induction or repression of RNA-binding proteins induced by TGFβ1. We further proposed to isolate and attempt to identify these TGFβ-regulated proteins. Our aims involve initially biochemical assays (eg gel-shift) and then cell-based (transfections) experiments.

Body

In our Statement of Work, we outlined 3 Specific aims encompassing 5 tasks. Portions of the first two aims were to begin in year one. Specific Aim 1 was to map the MMP-9 transcript's 3'untranslated region (UTR), for broad and then narrow regions critical to maintenance of transcript stability. Our first task was to begin by constructing several new plasmids with deletions of relatively large regions within the MMP-9 3'UTR. We have thus far designed and built 2 constructs: one with the entire 500 bp 3'UTR deleated leaving a 2000 bp coding region (2.0 kb fragment MMP-9); and one with only the 3'UTR left (0.5 kb fragment MMP-9). We have also made (or are in the process of making) constructs with the coding region plus the first 100 bp of the 3'UTR, the first 200 bp of the UTR, the first 300 bp of the UTR and the first 400 (out of approximately 500 bp) of the MMP-9 UTR. In this way, we will have constructs with the MMP-9 coding region covering 100 nucleotide streatches of the entire 3'UTR in a Bluescript vector to use for in vitro transcription.

In our Specific Aim 2, we proposed to analyze broad then smaller transcripts from constructs made in Aim 1 for TGFβ1-regulated protein-binding. We have acquired, grown and collected cytosolic and nuclear lysates (Marzluff et al, 1997) from 4 prostate cancer cell lines (DU145, PC-3, Tsu-Pr1, ND-1) with or without TGFβ1-stimulation. These lysates will be incubated with 3'UTR transcripts via RNA gel-shift to identify potential TGFβ1-regulated protein-bound regions (Bagga et al., 1999; Bagga and Wilusz, 1995).

Problems in Year 1: In the initial proposal, I had enlisted 2 graduate students, Ms. Li Qin and Ms. Sarah Holmes for these studies. Ms. Qin left my laboratory to pursue a graduate degree in Statistics and Ms. Holmes left to enter the Pharmacy Doctoral program. I have recruited one graduate student into the group (Ms. Andrea Grief), one undergraduate (Mr. Jeffery Lachowitzer) and one Pharm D student, Mr. William Fischer) to work on this project. In addition, I am in the process of recruiting a technician (to be paid out of the NIH COBRE grant described below). There was a lose of continuity and productivity after the departures of Ms. Qin and Holmes because I had to retrain (and am still training) my 3 new group members. In addition, because of the COBRE award to study MMPs in cancer, my laboratory will undergo extensive remodeling—a new tissue culture facility will be constructed this year. This facility will include the addition of two new tissue culture hoods, 2 new microscopes, incubators, and work bench areas. In addition, I will have a newly constructed dark room attached to the laboratory for processing of gel shift films. This remodeling and the asbestos removal which

must accompany it, will necessitate that the laboratory and cell culture be temporarily moved and closed part of this next year. I anticipate that the inefficiency associated with this moving will necessitate stretching this DOD award out over 4 years vs. 3.

Key Research Accomplishments

- Construction of plasmids with 100 bp deletions coving the MMP-9 3'UTR.
- Collection of cytosolic and nuclear extract from prostate cell lines

Reportable Outcomes

In 2000, I applied for an NIH RFA program grant along with a group of 5 other investigators. This grant award, called a C-O-B-R-E (Center of Biomedical Research Excellence) is set aside for EPSCoR state universitys to fund infrastructure and research proposals to enhance competitiveness within these states. This award was funded in February of 2001 and as a result, I will run a cell culture facility to test newly developed MMP inhibitors. Much of the background for my aspect of this grant came about as a result of this DOD award. The building renovations described above which will be of tremendous benefit to the aims of this DOD award, will be paid for from this COBRE money.

Conclusions

Our preparations thus far will allow us to run several gel-shifts in year 2. The implications are that these experiments may demonstrate regions within the UTR necessary for TGFβ1 regulation, if this regulation is through an enhanced protein binding or reduced binding, and if the proteins are cytosolic or nuclear. Constructs which appear to have altered protein binding after TGFβ treatment will be subcloned into mammalian expression vectors. We will transfect the prostate cancer cells with these vectors to determine if the TGFβ-induced increase in MMP-9 half-life at the levels of the RNA and protein products are altered with the 3'UTR deletions.

"So What Section"—Our experimental process is still in the foundation building stage; however with our constructs and cell lysates, we have the reagents to provide answers about how this critical MMP is regulated by a critical growth factors during prostate cancer metastasis.

References

Bagga P., Ford, L, Chen, F and Wilusz, J. the G-rich auxiliary downstream element has distinct sequence and position requirements and mediates efficient 3' end pre-mRNA processing through a trans-acting factor. Nucleic Acids Res. 23:1625-31, 1995.

Bagga, p and Wilusz, J. Northwestern screening of expression libraries. *In:* Methods in molecular biology, vol. 118: RNA-protein interaction protocols. ed. Susan Haynes Humana Press Inc. 1999.

Marzluff, W.F., Whitfield, M.L., Dominski, Z., and Wang Z-F. Identification of the protein that interacts with the 3' end of histone mRNA. *In:* mRNA Formation and Function, ed. Joel D. Richter. Academic Press 1997.

Appendices None